# Annual Short Report for the Southern Center on Environmentally Driven Disparities in Birth Outcomes

**Period covered by the report: 5/1/2010 – 4/30/2011**

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## Center Overview

The central mission of the Southern Center on Environmentally-Driven Disparities in Birth Outcomes is to determine how environmental, social, and host factors jointly contribute to health disparities. Specific aims of the Center are:

1. *To develop and operate an interdisciplinary children's health research center with a focus on understanding how biological, physiological, environmental, and social aspects of vulnerability contribute to health disparities;*
2. *To enhance research in children's health at Duke by promoting research interactions among programs in biomedicine, pediatric and obstetric care, environmental health, and the social sciences and establishing an infrastructure to support and extend interdisciplinary research;*
3. *To develop new methodologies for incorporating innovative statistical analysis into children's environmental health research and policy practice, with a particular emphasis on spatial, genetic and proteomic analysis;*
4. *To serve as a technical and educational resource to the local community, region, the nation, and to international agencies in the area of children's health and health disparities*; *and,*
5. *To translate the results of the Center into direct interventions in clinical care and practice*.

SCEDDBO leverages and promotes active partnerships among the Nicholas School of the Environment, the Duke University Medical Center, Trinity College of Arts and Sciences, and Duke's Children's Environmental Health Initiative, as well as the Durham County Health Department (DCHD), and the Lincoln Community Health Center (LCHC). The Center brings together the expertise of obstetricians, pediatricians, genetic epidemiologists, spatial statisticians, environmental scientists, social epidemiologists, social psychologists, geographers, and community organizations. SCEDDBO capitalizes on substantial ongoing commitments by Duke University to foster strong interdisciplinary research programs in environmental health sciences.

**Synthesis across SCEDDBO**. **Research Project A: Mapping Disparities in Birth Outcomes** provides population-level research on health disparities in birth outcomes. Spatially-linking 1.7 million birth records with environmental, social, and host factor data layers allows for population-level analysis of potential co-factors identified in both the clinical obstetrics **Research Project B: Healthy Pregnancy, Healthy Baby: Studying Racial Disparities in Birth Outcomes** and mouse model **Research Project C: Perinatal Environmental Exposure Disparity and Neonatal Respiratory Health** studies. The data from Research Project A is spatially linked in GIS to the data from Research Project B.

The neighborhood assessment undertaken in Research Project B provides important neighborhood-level environmental and social data to Research Project A. In addition, the environmental data developed for Research Project A works synergistically with the mouse

model work in Research Project C. For example, the air quality data from Research Project A is being used to further refine experimental dose design in Research Project C. In turn, results from Research Project C regarding experimental effects of multiple environmental agents on fetal growth restriction and postnatal somatic and lung development help point to locations in North Carolina where we are looking more closely at air quality impacts on birth outcomes in Research Project A.

Thus Research Project A is an epidemiological study, while Research Project B is a complementary clinical obstetrics project. Both projects focus on how combined environmental, social, and host factors shape disparities in birth outcomes. Research Project B also allows for additional host factor analysis. Research Project C uses a mouse model system to explore how disparities in exposure and response to exposure initiate and/or enhance disparities in birth outcomes and subsequent neonatal respiratory health. Like Research Projects A and B, Project C explores the effects of *combined* environmental exposures to prototypical air pollutants common in North Carolina (particulate matter and ozone), as well as social stress, on fetal growth restriction, neonatal somatic growth, and subsequent lung development and function.

The synergy among the research projects is facilitated by the GIS and Statistical Analysis (GISSA) Core. The GISSA Core allows for data analysis of the very large amount of data through the use of high-end GIS applications in combination with Bayesian spatial hierarchical modeling and other advanced spatial statistical approaches, thus permitting multi-level analysis. Research Projects A and B both apply a Bayesian spatial hierarchical modeling approach to capture uncertainties in pregnancy outcomes and to elucidate the contributions of economic, sociocultural, and environmental stressors on health disparities in pregnancy outcomes. State-of-the-art GIS methods allow for sophisticated spatial statistical analyses at highly resolved spatial scales.

The GISSA Core also provides the analysis of the biological response and genetic data generated in Research Projects B and C. The rich source of social, environmental, and host data in Project B, coupled with sophisticated statistical genetic approaches for identifying gene-gene and gene-environment interactions, provides the opportunity to make important discoveries of how these higher order interactions may be working together to promote or prevent adverse birth outcomes. By serving as a central clearinghouse for statistical analysis, the GISSA Core tracks outcomes in each project and uses these discoveries to guide the analysis in each of the other projects.

The Community and Outreach Translation Core (COTC) facilitates the communication of findings from our large-scale study and future more-focused investigations. The COTC supported the implementation of the neighborhood assessment undertaken in Research Project B and has helped to communicate the results of the assessment to community partners. In addition, the COTC draws on the GISSA Core to develop materials that communicate the results of the research projects in formats and applications that are immediately accessible to the lay public.

SCEDDBO is characterized by significant synergies among center components. To provide concrete examples of how the work of the center is moving forward in a collaborative way, here we highlight four areas: air pollution, social context of environmental stress, the Community Assessment Project, and statistical methods development. We provide summaries in this center overview; additional details can be found in the individual center component write-ups.

*Air Pollution*. To investigate the relationship of air pollution exposure and pregnancy outcomes, we have examined air pollution in all three projects. In Projects A and B, we have used criteria air pollutant data from the EPA AQS monitoring network, as well as CMAQ and FUSED modeling data. In addition, we have recently obtained highly resolved air toxics data. These

data have been spatially linked to the births in both Projects A and B. In addition, we have created a road proximity measure which can be used in both Projects A and B. The road proximity measures allow us to consider a relatively simple metric for assessing risk of exposure to air pollution, specifically traffic-related air pollution which includes particulate matter and diesel exhaust, both of which are being investigated within Project C. We have already published several manuscripts on the relationship between air pollution and pregnancy outcomes and anticipate several more in Year 5. We are also preparing a manuscript that synthesizes the air pollution work done across projects to be submitted during year 5.

*The Social Context of Environmental Stress.* We continue to work toward synthesis across all three projects. We have been able to combine our knowledge of the pregnant women in Project A with our rich data from the pregnant women in Project B. With our access to the North Carolina Detailed Birth Record (DBR) in Project A, we have been able to link participants in Project B with their birth certificate data. Using maternal and infant identifying information, including name, place, and date of birth, we have been able to link 1349 (99.0%) participants who completed the study and had a live birth by December 31, 2009 and 96 (79.3%) participants that were lost-to-follow-up but with an expected delivery date on or before December 31, 2009. This linkage will allow us to examine multiple questions including racial residential segregation, residential mobility, and maternal medical complications.

Additionally, the effects of resource deprivation suggested by findings in Projects A and B prompted Project C to add a resource deprivation (nesting restriction) component in order to test the proof-of-principle that the combination of multiple stressors/environmental contaminants may affect health even when the individual exposures do not.

*Community Assessment Project/Built Environment.* An important measure of potential environmental stress is the built environment. Our Community Assessment Project assessed built environment variables for over 17,000 tax parcels, including the home addresses of over 40% of the participants in the Healthy Pregnancy, Healthy Baby Study (SCEDDBO Project B). Analyses of the built environment data are underway. Seven scales (housing damage, property disorder, security measures, tenure, vacancy, violent crime and nuisances) have been constructed at five levels of geography (census block, primary adjacency communities, census block group, census tract, and city-defined neighborhoods). The continuous and categorical scale variables have been merged with the Durham birth records (Project A) and with the clinical OB participants' records (Project B), which enables multiple analyses of the relationships among the built environment, psychosocial health, and pregnancy outcomes. The second wave of data collection is planned for year 5.

*Statistical Methods Development*. We are pursuing three projects that capitalize on combining information in the data for Project A and Project B. The first project is to utilize the fine detail in Project B data to improve analyses involving Project A data.

The second project is to use the Project B data to check the sensitivity of conclusions from Project A analyses to potential unmeasured confounding. This is accomplished by comparing the findings from models fit using Project A data with the findings from parallel models fit using Project B data that control for additional relevant variables available only in Project B. If the associations found in the Project A models remain robust after including the potential confounders from Project B, our confidence in the conclusions increases. We are working on methods that perform such tests in a principled, model-based manner. In a related project, we also are checking the sensitivity of conclusions from Project A analyses to possible measurement errors in the data. For example, educational attainment variables for mothers in the intersection of Project A and Project B are quite different on the two datafiles. We treat Project B education values as truth—since we are more confident in their accuracy—and

replace the Project A education values with this new truth. For mothers in the intersection of the datasets, we then can re-run analyses to see if results change dramatically. We also are working on imputing corrected values of education for the entire Project A data.

The third project is to explore factors that affect maternal blood pressure during pregnancy. This project involves combining pollution data from Project A with other data from Project B. We consider a variety of statistical approaches for this project, including latent trajectory and sparse functional data models. In the latter approach, we introduce a low-dimensional set of latent factors to predict blood pressure curves. Environmental, social, and genetic factors are used to help explain variation in the blood pressure trajectories. Our ultimate goal is to link these predicted trajectories to birth outcomes; for example, women with monotonically-increasing blood pressure trajectories may exhibit poorer birth outcomes than women with U-shaped curves. Methodological extensions include joint modeling of blood pressure and air pollution trajectories via structural equation models.

**Project Title: Research Project A: Mapping Disparities in Birth Outcomes**
**Investigators: Marie Lynn Miranda (PI), Alan Gelfand, Pamela Maxson, Evan Myers**

The central objective of Project A is to determine whether and to what extent joint exposures to socioeconomic and environmental stressors contribute to racial and ethnic health disparities in poor pregnancy outcomes. Using a geographically-based nested study design moving from analysis of births for the entire State of North Carolina to six demographically and geographically distinct counties to a single health center and state-of-the-art Geographic Information Systems applications with Bayesian spatial hierarchical modeling and other advanced spatial statistical approaches, the specific aims are to:

1. Spatially link detailed birth record, fetal death certificates, socioeconomic, environmental, tax assessor, community-based, and clinical obstetric data at highly resolved scales for the State of North Carolina from 1990-2003;
2. Refine the concept of fetal growth restriction by a) developing a joint distribution for birthweight and gestation using bivariate modeling for live births and fetal deaths – both separately and jointly, and b) defining it in terms of fetal and infant mortality, rather than percentile cut points; and
3. Determine whether and to what extent differential exposures to both environmental and social stressors help explain health disparities in fetal growth restriction among a) African-American women compared to Non-Hispanic white and Hispanic women, b) Older African-American women compared to younger African-American women, c) Hispanic women compared to Non-Hispanic white and African-American women, and d) Foreign born Hispanic women compared to US born Hispanic women.

**Summary of Accomplishments**

We have linked the detailed birth record data to USEPA $PM_{10,}$ $PM_{2.5,}$ and ozone monitoring data in order to study the impact of maternal exposure to air pollution on birth weight. We are especially focused on refining exposure metrics to most effectively characterize meaningful exposures, as well as to capture any windows of vulnerability. Significant progress has been made on the relationship between birth outcomes and exposure to particulate matter and ozone separately, and the current focus is determining how to characterize joint exposure to both particulate matter and ozone.

A substantial amount of effort this year has been devoted to a novel project concerned with connecting the built environment to adverse pregnancy outcomes. Built environment data has

been collected under the Community Assessment Project and, after preliminary analysis has focused on spatial layers capturing four primary attributes of the built environment: housing damage, property disorder, tenure, and vacancy. Connection has been made to pre-term birth and low birth weight.

Our project on racial residential segregation has now seen the completion of one paper which enables quantification of racial exposure/isolation at finer spatial scales within SMSA's. Such a measure can be connected to measures of social and economic disadvantage at these scales to gain insight into how racial residential segregation has manifested itself across urban landscapes. In turn, this promises to reveal key insights into how to think about the spatial aspects of the social factors influencing health disparities.

**Future Activities**

We plan to continue working on each of the areas described in the progress report/summary of accomplishments section. Achieving a better understanding of exposure to air toxins, particularly particulate matter and ozone, is a central focus of our future efforts. We recently began the process of linking participants in Project B with their associated birth certificate record. This linkage will not only allow us to explore issues of data accuracy in the detailed birth record, but also allow us to begin implementing the methods of synthesizing categorical data.

**Publications**


Anthopolos, R, James, SA, Gelfand, AE, and Miranda, ML**.** A Spatial Measure of Neighborhood-level Racial Isolation Applied to Low Birthweight, Preterm Birth, and Birthweight in North Carolina. Forthcoming. *Spatial and Spatio Temporal Epidemiology.*

Berrocal, VJ., Gelfand, AE., Holland, DM., Burke, J., Miranda, ML.. On the Use of a $PM_{2.5}$ Exposure Simulator to Explain Birthweight. 2011, *Environmetrics*. 22(4), 553-571.

Chang HH, Reich BJ, Miranda ML. Time-to-Event Analysis of Fine Particle Air Pollution and Preterm Birth: Results from North Carolina 20001-2005. Forthcoming. *American Journal of Epidemiology.*

Gray, S, Edwards, S and Miranda, ML**.** Assessing Exposure Metrics for PM and Birth Weight Models. 2010. *Journal of Exposure Science and Environmental Epidemiology:* 20(5): 469-477. PMCID: PMC2889210.
Gray, SC., Gelfand, AE., Miranda, ML**.** Hierarchical Spatial Modeling of Uncertainty in Air Pollution and Birth Weight Study. Forthcoming. *Statistics in Medicine*.

Miranda, ML, Anthopolos, R, and Edwards, SE. Seasonality of Poor Pregnancy Outcomes. Forthcoming. *North Carolina Medical Journal.*

Miranda, ML, Edwards, SE, and Myers, ER., Adverse Birth Outcomes among Nulliparas versus Multiparas. Forthcoming. *Public Health Reports*.

Miranda, ML, Swamy, G, Edwards, S. Maxson, PJ, Gelfand, A, and James. S. Disparities in Maternal Hypertension and Pregnancy Outcomes: Evidence from North Carolina, 1994-2003. 2010. *Public Health Reports,* July/August 125(4):579-587. PMCID: PMC2882609.

Schwartz, S, Gelfand, A, Miranda, ML**.**.Joint Bayesian Analysis of Birthweight and Censored Gestational Age using Finite Mixture Models. 2010 *Statistics in Medicine,* 20; 29(16):1710-1723.

Swamy, G. Edwards, S. Gelfand, A, and Miranda, ML. Maternal Age, Birth Order, and Race: Differential Effects on Birthweight. 2010 *Journal of Epidemiology and Community Health*. Published Online First: 15 November 2010. doi:10.1136/jech.2009.088567.

**Supplemental Keywords**: Data fusion, meta analysis, disparities, spatial disaggregation, spatial interpolation, spatial modeling, racial residential segregation

**Project Title: Research Project B: Healthy Pregnancy, Healthy Baby: Studying Racial Disparities in Birth Outcomes**
**Investigators: Redford Williams (PI), Allison Ashley-Koch, Richard Auten, Pamela Maxson, Marie Lynn Miranda, Jerome Reiter, Geeta K. Swamy**

The central objective of the Healthy Pregnancy, Healthy Baby Study is to determine how the interaction of environmental, social, and host factors contributes to disparities in birth outcomes between African-American and white women in the American South. There are four specific aims:

1. Conduct a cohort study of pregnant women in Durham, NC designed to correlate birth weight, gestation, and birth weight x gestation with environmental, social, and host factors;
2. Develop community-level measures of environmental and social factors by inventorying neighborhood quality and the built environment in partnership with local community groups;
3. Create a comprehensive data architecture, spatially resolved at the tax parcel level, of environmental, social, and host factors affecting pregnant women by linking data from the cohort study and neighborhood assessments with additional environmental and socioeconomic data; and
4. Determine whether and to what extent differential exposures explain health disparities in birth outcomes by applying innovative spatial and genetic statistical methods to:
    a. Identify environmental, social, and host factors that cluster to predict birth outcomes in the entire sample,
    b. Determine whether these clusters are more or less present in African-American versus white populations and quantify the proportion of health disparities explained by differences in cluster frequency, and
    c. Identify environmental, social, and host factors that cluster to predict birth outcomes within the African-American and white sub-samples and compare these clusters across racial groups.

**Progress Report/Summary of Accomplishments**

As of 4/1/2011, 1889 women have been enrolled in the study. Demographic data indicate that we are successfully recruiting women who are most at risk for adverse pregnancy outcomes, particularly low-income, low educational attainment, and non-Hispanic black women.

We have been highly successful in collection of participant-level data as well as biological samples, with greater than 90% attainment of maternal blood sample for genetic and environmental analyses. Collection of cord blood and placental samples, which began in June 2007, has also been successful with approximately 944 delivery samples collected.

All maternal data is georeferenced (i.e., linked to the physical address of the mother) using Geographic Information System (GIS) software. The Healthy Pregnancy/Healthy Baby Study also includes an in-depth neighborhood assessment designed to capture both built environment and community-level social stressors and community resources. The cohort study and neighborhood assessment data are spatially linked to extensive environmental and demographic data at a highly resolved spatial scale.

*Genetic Data and Analysis.* To date, we have generated genotypes on approximately 1600 blood samples from pregnant women. We have genotyped 412 Single Nucleotide Polymorphisms (SNPs) in fifty-two genes.

*Psychosocial Indicators.* Analyses have been completed on psychosocial influences on birth outcomes. The relationships among pregnancy intention, psychosocial health, and pregnancy outcomes have been examined, with a paper accepted. In addition, we are examining pregnancy intention, behavioral choice, and environmental exposures. The influences of psychosocial health and smoking status have been studied, and a paper has been submitted. In order to reduce the number of psychosocial variables, cluster analysis has been performed, resulting in three distinct clusters of women. Cluster analysis on the personality indices were also performed. A resulting paper presented at the Society of Behavioral Medicine meeting reported that women with an adverse personality profile were more likely to express several psychosocial risk factors (e.g., increased depressive symptoms, increased unwanted pregnancy) and had a six-fold higher rate of preterm (<32 weeks) delivery than women with a resilient profile.

*Maternal Medical Complications.* Fetal health is not only individually determined, but is also influenced by maternal health and well-being. This past year, we have begun to examine maternal outcomes, as well. In particular, we have begun to focus on hypertensive disorders during pregnancy.

*Statistical Methods Development.* We developed several new statistical methodologies designed to improve analysis of the Project B data, as well as to advance statistical analysis more broadly. First, we developed and implemented methods for finding important predictors in quantile regression when there are a very large number of covariates. These methods adapted the lasso and elastic net penalties for quantile regression. We applied the methods on a mid-study sample of women to uncover a previously unreported interaction: women who smoke and who have high blood lead levels tend to have babies with lower birth weights.

Second, we developed and implemented methods for using factor analysis models in the context of quantile regression. The investigative team believes that many of the predictors can be grouped into underlying factors. For example, the Project B data contain several variables that measure maternal stress, and arguably we should connect birth outcomes to the underlying factor of stress rather than its individual indicators. As another example, the data contain several imperfect indicators of smoking status, and we would like to connect birth outcomes to the underyling factor of true smoking status. We implemented the model on a mid-study sample of women from Project B, and we found that the smoking factor was a strong predictor of low birth weight.

Third, we developed and implemented methods for accounting for mid-study changes in measurement scales. These methods were needed because the Project B investigators switched assay labs for measuring blood levels of heavy metals midway through data collection in order to take advantage of finer measurement scales. Exploratory analysis indicated that the distributions of levels for several exposures were markedly different across the labs, so that analyses based on a simple concatenation of the two labs' data would be biased. Using the second lab scale as the standard, so that effectively measurements before the lab switch are

treated as missing, we developed general purpose methodology for imputing plausible values of the missing exposure measurements. The methods are based on assumptions about the relative ranks of measurements in the two scales, e.g., a measurement in the 10$^{th}$ percentile in one scale should be at the 10$^{th}$ percentile in the other scale. We implemented this methodology on the Project B data to provide the investigative team with improved data product.

We also developed a Bayesian growth mixture model to jointly examine the associations between longitudinal blood pressure measurements, preterm birth (PTB), and low birthweight (LBW). The model partitions women into distinct classes characterized by a mean arterial pressure (MAP) curve and joint probabilities of PTB and LBW. Each class contains a unique mixed effects model for MAP with class-specific regression coefficients and random effect covariances. To account for the high correlation between PTB and LBW, we introduce a bivariate probit model within each class to capture residual within-class dependence between PTB and LBW. The model permits the association between PTB and LBW to vary by class, so that for some classes, PTB and LBW may be positively correlated, while for others, they may be uncorrelated or negatively correlated. We also allow maternal covariates to influence the class probabilities via a multinomial logit model. For posterior computation, we propose an efficient Markov chain Monte Carlo algorithm that combines full-conditional Gibbs and Metropolis steps. We apply our model to a sample of 1027 women enrolled in the Healthy Pregnancy, Healthy Baby Study, a prospective cohort study of host, social, and environmental contributors to disparities in pregnancy outcomes.

**Future Activities**

In the next year, we will focus on data analysis and further statistical methods innovation. Our primary interest is in bringing these two pieces together. The statistical methods innovation is driven by the needs of our data analysis and thus will continue to explore means to reduce the dimensionality of the genetic and other data, as well as impute missing data. Our overall goal is to identify complex interactions amongst the three sides of the triangle we hypothesize influence pregnancy outcomes: host, social, and environmental contributors.

**Publications**

Burgette, LF, Reiter, JP. Multiple Imputation via Sequential Regression Trees. 2010. *American Journal of Epidemiology*, *172*, 1070-1076.

Burgette, LF, Reiter, JP, and Miranda, ML. Exploratory Data Analysis for Quantile Regression: An Application to Adverse Birth Outcomes. Forthcoming. *Epidemiology.*

Burgette, LF, Reiter, JP. Modeling Adverse Birth Outcomes via Confirmatory Factor Quantile Regression. Forthcoming. *Biometrics*.

Burgette, LF, Reiter, JP. Nonparametric Bayesian Multiple Imputation for Missing Data due to Mid-Study Switching of Measurement Methods. Forthcoming. *Journal of the American Statistical Association.*

Maxson PJ, Miranda ML "Pregnancy Intention, Demographic Differences, and Psychosocial Health." Forthcoming. *Journal of Women's Health.*

Miranda ML, Edwards S, Maxson PJ. Mercury Levels in an Urban Pregnant Population in Durham County, North Carolina. 2011. *International Journal of Environmental Research in Public Health,* Mar 8(3):698-712.

Miranda, ML Edwards, SE Swamy, G Paul, C and Neelon.B. Blood Lead Levels among Pregnant Women: Historical versus Contemporaneous Exposures. 2010. *International Journal of Environmental Research and Public Health* 7(4): 1508-1519.

Neelon B, Swamy GS, Burgette LF, and Miranda, ML. A Bayesian Growth Mixture Model to Examine Maternal Hypertension and Birth Outcomes. 2011. *Statistics in Medicine*, *30*, 2721–2735.

Schwartz, S, Li, F, and Reiter, JP. Sensitivity Analysis for Unmeasured Confounding in Principal Stratification. Forthcoming. *Statistics in Medicine*.

Swamy, GK, Garrett, ME, Miranda, ML, Ashley-Koch, AE. Maternal Vitamin D Receptor Genetic Variation Contributes to Infant Birthweight among Black Mothers. 2011. *American Journal of Medical Genetics Part A, 155,* 1264-71.

**Supplemental Keywords**

Pregnancy, preterm birth, low birth weight, racial disparity, African American, environmental stressors, gene-environment interactions, psychosocial stressors, genes, single nucleotide polymorphisms, genetic admixture

**Project Title: Research Project C: Perinatal Environmental Exposure Disparity and Neonatal Respiratory Health**
**Investigators: Richard L. Auten (P.I.), W. Michael Foster**

The specific aims of Project C are:

1. To determine whether maternal exposure to airborne particulates (PM) and/or ozone (1$^{st}$ hit) restricts fetal growth and/or postnatal growth, and impairs lung development/function in newborn mice;
2. To determine whether PM and/or ozone exposure ‘re-programs’ maternal inflammatory responses;
3. To determine whether postnatal (2$^{nd}$ hit) ozone exposure further impairs postnatal somatic and lung development/function following maternal PM and/or ozone exposures;
4. To determine whether genetic or developmental susceptibility to airway hyperreactivity exacerbates maternal and/or postnatal exposure effects on postnatal somatic and lung development/function.

**Summary of Accomplishments**

1. Aim 4 was focused on genetic susceptibility. To determine which pathways are important to transducing maternal air pollutant exposures to adverse effects on fetuses and newborns, we have conducted studies in mice lacking the Tlr4 gene, a key innate immune response receptor previously shown to be important to acute ozone induced airway hyperreactivity in adult mice. Our studies have shown that the placental, fetal lung, and fetal brain cytokines that were induced by maternal diesel inhalation are in many cases dependent on maternal+fetal Tlr4 signaling. In particular we have found that the maternal diesel inhalation/instillation effects on IL-1$\beta$, IL-6, KC, TNF$\alpha$, and eotaxin responses in the placenta, and IL- $\beta$, TNF$\alpha$, and MIP-1, and RANTES in the lung are dependent on Tlr4.
2. Increasing evidence points to epigenetically mediated heritable effects of environmental pollutant exposures on health outcomes. We have conducted studies using the diesel particle described in the prior Progress Report to test this concept. Maternal diesel exposure increases the vulnerability of offspring to inflammatory airway challenge with nebulized endotoxin, a ligand for Tlr4. Studies done in collaboration with J. Hollingsworth and D. Brass suggest these increased susceptibilities are epigenetically

mediated, with inheritance of the diesel exposure effect to the F3 generation. Current studies are aimed at identifying specific molecular pathways that may be responsible.

3. Since the last reporting period, we have conducted additional studies on the effects of resource deprivation (nest/housing restriction) in combination with maternal pollutant (diesel) exposure and found that the combination impairs postnatal weight gain and worsens the response to inflammatory endotoxin challenge. We did not observe effects on airway hyperresponsiveness, but do not expect this without the contribution of post-natal ozone exposure. These studies suggest that the combination of sub-clinical chemical and non-chemical stressor exposures have important effects on lung susceptibility to inflammatory challenge in offspring at a juvenile stage of development. The extension of our studies to include these components is funded by a Duke Integrative Brain Sciences incubator award that was competitively renewed for FY11-12.
4. We are finishing our studies on the neural contributions towards ozone induced airway hyperresponsiveness and a manuscript is in preparation.
5. Since oxidative stress is an important pathway implicated in ozone induced asthma in children, we sought to determine whether an asthma susceptibility gene, NQO1 (NAD(P)H quinone oxidoreductase-1) was also important in conferring airway hyperresponsiveness in our animal model. We have completed studies showing that *Nqo1* null mice are completely protected from the effects of neonatal ozone exposure on prolonged airway hyperreactivity that persists to adulthood. This strongly implicates the oxidative stress responses during early life in the development of later airway hyperreactivity. We are repeating some of the pivotal experiments to confirm this. In contrast with our studies of combined maternal diesel and postnatal ozone exposure, we did not find substantial effects of ozone, with or without *Nqo1* knockout, on alveolar development.

**Future Activities**

1. We are continuing to determine the contribution of combined chemical and non-chemical perinatal stressors on respiratory and neurocognitive development of offspring. We are extending our studies using the Tlr4 null mice to determine the contribution of either maternal innate immune responses or fetal/neonatal immune responses to the adverse effects of the combined stressors on lung and brain development.
2. The epigenetic contributions will be studied in more detail by evaluating particular molecular pathways in pulmonary macrophages which appear to be critical to the effects on airway hyperreactivity in mice born to dams exposed to diesel inhalation.

**Publications**


1. Auten RL and Foster WM. Biochemical Effects of Ozone on Asthma Development. *BiochimicaetBiophysicaActa*, in press.
2. Auten RL, Gilmour MI, Potts EN, Mason SN, Foster WM. Maternal diesel inhalation increases airway hyperreactivity in ozone exposed offspring. Submitted.
3. Auten RL, Mason SN, Potts EN, Chitano P, Foster WM. Neonatal murine ozone exposure induces neutrally mediated airway hyperreactivity persisting to adulthood. In preparation.


**Supplemental Keywords**


Epigenetic, innate immunity, Nqo1